Br. J. Pharmacol. (1992), **106**, 61–66

Combined dose-ratio analysis of cholecystokinin receptor antagonists, devazepide, lorglumide and loxiglumide in the guinea-pig gall bladder

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1 Interactions between cholecystokinin octapeptide (CCK-8) and CCK_A-receptor antagonists derived from benzodiazepines (devazepide) and glutamic acid (lorglumide and loxiglumide) have been examined in an improved bioassay using the guinea-pig, isolated, gall bladder preparation.

2 The presence of CCK_B-receptors in the assay was provisionally-ruled out on the basis of the low potency of pentagastrin in the assay. By applying analyses of both agonism and antagonism, pentagastrin was shown to behave as a partial agonist at the CCK_A-receptor.

3 Devazepide, lorglumide and loxiglumide behaved as simple competitive antagonists of CCK_A-receptors and pK_B values of 9.98, 7.59 and 7.07 were estimated, respectively.

4 Application of a combined dose-ratio analysis to the interactions between CCK-8 and combinations of devazepide/lorglumide and devazepide/loxiglumide indicated that these molecules behave as syntopic, competitive, antagonists at the CCK_A-receptor.

5 We conclude that the guinea-pig gall bladder assay contains a homogeneous population of CCK_A-receptors and offer an explanation for the differences between our results and those obtained recently by Maubach *et al.* (1991) which were taken as preliminary evidence for CCK_A-receptor heterogeneity.

Keywords: Receptor, cholecystokinin; muscle, smooth, gall bladder; pentagastrin; devazepide; lorglumide; loxiglumide

Introduction

The pharmacological classification of cholecystokinin (CCK)/gastrin receptors as CCK_A and CCK_B was proposed on the basis of the behaviour of selective agonists and antagonists in both radioligand binding studies and functionally-intact bioassays (see Woodruff & Hughes, 1991). Among the most frequently-used antagonists for testing and developing this classification are the benzodiazepine derivative, devazepide (Evans *et al.*, 1986) and the glutamic acid derivatives lorglumide (Makovec *et al.*, 1987) and loxiglumide (Setnikar *et al.*, 1987). Devazepide, lorglumide and loxiglumide are reported to be selective, competitive, antagonists for CCK_A-receptors with pK_D values of ~ 10.1, 7.7 and 6.5, respectively as compared with pK_D values at CCK_B-receptors of ~ 6.6, 5.6 and 5.0, respectively (Setnikar *et al.*, 1987; Lotti & Chang, 1989). These pK_B values have been estimated on the assumption that the agonist-antagonist interactions are competitive. If these ligands are to be used to prove the homogeneity of hormone receptors, then the assumption that the competitive behaviour is due to mutual exclusivity at a common site, so called syntopic behaviour, must be made.

However, 'competitive' behaviour can be found for example in bioassays involving high efficacy agonists and functional antagonists and in ligand binding assays involving agonists and allosteric antagonists.

There are marked differences in chemical structure and physico-chemical properties between the benzodiazepine and glutamic acid derivatives and the peptide hormone itself. Consequently, it seemed pertinent to question whether these ligands compete for the same site at the CCK_A-receptor. We have applied a combined dose-ratio analysis (Paton & Rang, 1965; Black *et al.*, 1986; Shankley *et al.*, 1988) which, as far as we are aware, provides the only currently available test for behaviour consistent with a syntopic, competitive mechanism of action. The experiments were performed on the guinea-pig gall bladder assay which has conventionally been considered to contain a single population of CCK_A-receptors. We found that it was necessary to improve the existing assay so that it met the generally-accepted criteria for quantitative analysis (Furchgott, 1972; Black & Shankley, 1985). However, during the course of these studies Maubach *et al.* (1991) presented data which, they concluded, provided preliminary evidence for CCK_A-receptor heterogeneity in the guinea-pig gall bladder. Our findings are discussed in the light of their conclusions.

Methods

Guinea-pig gall bladder assay

The assay was based on the method described by La Morte *et al.* (1981). In brief, four longitudinal strips of smooth muscle were dissected from each gall bladder taken from male Dunkin-Hartley guinea-pigs (250–500 g) and suspended in 20 ml organ baths maintained at 29°C ± 0.5 in modified (low Ca^{2+}) Krebs-Henseleit solution (mM: Na^+ 143, K^+ 5.9, Ca^{2+} 0.5, Mg^{2+} 1.2, Cl^- 128, HPO_4^{2-} 1.2, SO_4^{2+} 1.2, D-glucose 10, HCO_3^- 25) and gassed with 95% O_2 and 5% CO_2. Following the application of a single 1 g load, the tissues were allowed to relax until a stable baseline was produced. Tension, expressed in grams, was continuously recorded with an isometric transducer.

Experimental protocols

A stable baseline was achieved after an initial 20–40 min relaxation period at which time the bathing solution was replaced and tissues incubated for a further 60 min period in

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the absence of an antagonist or appropriate vehicle. In preliminary studies it was found, following a primary CCK-8 concentration-effect (E/[A]) curve, that there was a residual degree of CCK_A-receptor stimulation even though the tissue had been subject to multiple washes (6 washes at 5 min intervals). Therefore, only a single, cumulative, agonist E/[A] curve was obtained in each tissue. Experiments were allocated to a randomised block design so that 4–8 replicates were obtained for each treatment group.

Data analysis

Logistic curve-fitting E/[A] data from individual preparations were fitted to a general logistic function to provide estimates of the midpoint slope parameter (p), midpoint location ($\log[A_{50}]$) and upper asymptote (α) of the curves. These parameters, expressed as mean ± s.e.mean, were used for subsequent analysis and display of data (see Black & Shankley, 1985 for details).

Competitive analysis Competitive analysis was performed according to the procedure described previously (Black *et al.*, 1985a). When no significant differences in values of p and α were found by one-way ANOVA, then the $\log[A_{50}]$ values in the absence and presence of antagonist (B) were directly fitted to the following derivation of the Schild equation,

$$\log[A_{50}]_B = \log[A_{50}] + \log\left(1 + \frac{[B]^b}{10^{\log K_B}}\right).$$

When the Schild slope parameter (b) was not significantly different from unity, then the data were re-fitted with b constrained to unity so that the antagonist equilibrium dissociation constant could be estimated as $\log K_B \pm$ s.e. For purposes of display, conventional Schild plots have been constructed with slopes of unity which intersect the abscissa scale at the pK_B calculated by the method above.

Combined dose-ratio analysis This analysis was performed according to the procedure developed by Shankley *et al.* (1988). In brief, when two antagonists act syntopically, that is, at the same site, then their combined dose-ratio is given by:

$$r_{(B+C)} = r_B + r_C - 1$$

where r_B and r_C are the dose-ratios obtained independently in the presence of the antagonists B and C, respectively. This relationship can be re-written in terms of the experimentally-estimated $\log[A_{50}]$ values,

$$S_A = \log[A_{50}]_{B+C} - \log([A_{50}]_B + [A_{50}]_C - [A_{50}]),$$

where S_A is the test statistic for the model which will have a value of zero when the data comply with the model. Similarly, when two antagonists act independently, that is, allotopically, their combined dose ratios multiply,

$$r_{(B+C)} = r_B.r_C$$

This relationship may also be expressed in terms of $\log[A_{50}]$ values,

$$S_M = \log[A_{50}]_{B+C} - \log[A_{50}]_B - \log[A_{50}]_C + \log[A_{50}],$$

so that S_M, the test statistic, will have a value of zero when the data complies with this model.

Analysis of agonism The agonism expressed by pentagastrin was analysed by direct model-fitting of the concentration-effect curve data to the following equation which describes the behaviour of an agonist in a single receptor-effector system (Black & Leff, 1983):

$$E = \frac{E_M[A]^n\tau^n}{(K_A + [A])^n + [A]^n\tau^n}$$

The fit was performed using the BMDP derivative-free, non-linear regression, programme (Dixon, 1990) on a VAX 3200 computer. The parameters for the maximum effect (E_M) and the slope parameter of the transducer function (n) were constrained to numeric values (see Results) and estimates made of the equilibrium dissociation constant (K_A) and the operational efficacy (τ) (for further details see Black *et al.*, 1985b).

Compounds

Compounds were obtained and prepared as follows: the sulphated octapeptide of cholecystokinin (CCK-8) and pentagastrin were obtained from Cambridge Biochemicals Ltd., UK. CCK-8 was dissolved in 10% absolute ethanol to provide a final stock solution of 2 mM concentration. Pentagastrin was dissolved in 100% dimethylformamide (DMF) to give a 100 mM stock. Devazepide, also known as L364718 (a gift from Merck, Sharpe & Dohme Ltd, U.S.A.), was dissolved in DMF to give a 0.12 mM stock concentration. Lorglumide (CR1409) and loxiglumide (CR1505) (Rotta Spa, Milan) were dissolved in an aqueous solution of NaOH (pH = 8) to give a 12 mM stock concentration. All compounds were subsequently diluted in distilled water. The maximum volume of DMF and distilled water added to any one 20 ml organ bath was 20 µl and 500 µl, respectively. Neither the vehicles nor the antagonists were found to produce significant effects on baseline tone.

Results

Analysis of CCK-8 concentration-effect (E/[A]) curve data in guinea-pig gall bladder

Assay problems were encountered when cholecystokinin octapeptide (CCK-8) was used as agonist on the guinea-pig gall bladder assay prepared according to the method of La Morte *et al.* (1981) who used it for studying the effects of histamine. Although concentration-dependent responses were obtained, the assay was prone to agonist-induced spontaneous activity and, as shown previously (Nieber *et al.*, 1988), unstable response profiles (Figure 1a). This behaviour made the determination of precise response levels uncertain. In addition, an unacceptably high variation was found in the shape and location ($\log[A_{50}]$) of the E/[A] curves (Figure 1a).

The spontaneous activity was eliminated by reducing the temperature from 37°C to 29°C and by lowering the $[Ca^{2+}]$ from 2.5 mM to 0.5 mM (Figure 1a). A reduction in variability was obtained by the use of very small (3 × 1 mm) strips cut from the body of the gall bladder (Figure 1a). These changes had the effect of reducing the standard deviation about the mean $\log[A_{50}]$ from 0.79 to 0.39 ($n = 21$, $n = 24$, respectively) and increasing the potency of CCK-8 by almost one log unit. Moreover, under the improved assay conditions the E/log[A] curves were consistently monotonic and symmetrical around the $\log[A_{50}]$. Therefore, it was possible, objectively, to analyse the data by fitting it to the three-parameter logistic function (see Methods) to obtain the parameter values like those shown in Table 1.

Analysis of pentagastrin concentration-effect (E/[A]) curve data in guinea-pig gall bladder

CCK-8 is reported to be a powerful stimulant of not only CCK_A-receptors but also CCK_B-receptors (Lotti *et al.*, 1986). Therefore, it was possible that the analysis of the antagonists could be confounded by the presence of CCK_B-receptors which might also be coupled to smooth muscle contraction in the gall bladder. This was investigated with pentagastrin as agonist because it is a selective agonist with nM potency in those tissues classified as containing CCK_B-receptors (Lotti *et al.*, 1986) but only µM potency in those now classified as

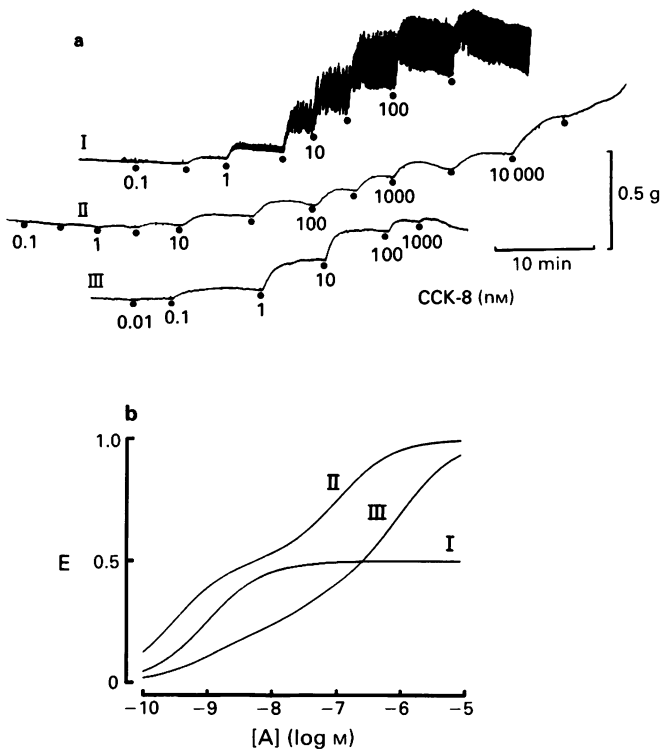

Figure 1 Development of the guinea-pig gall bladder assay. (**a**) Experimental traces showing cholecystokinin octapeptide (CCK-8) E/[A] curves obtained by cumulative dosing. Traces showing, (I) spontaneous activity observed when the assay was prepared according to La Morte *et al.*, 1981, (II) a complex curve following the reduction of both temperature, from 37°C to 29°C, and $[Ca^{2+}]$ from 2.5 mM to 0.5 mM and (III) a monotonic curve following the additional reduction in the size of the preparation (see Results for details) (**b**) Simulations showing theoretical E/[A] curves obtained from a mathematical model which describes the summation of effects from multiple units of tissue within a single bioassay preparation. Each unit is assumed to produce E as a simple rectangular hyperbolic function of [A], so that the total effect is given by,

$$E = \frac{\alpha_1.[A]}{[A_{50}]_1 + [A]} + \frac{\alpha_2.[A]}{[A_{50}]_2 + [A]} + \frac{\alpha_2.[A]}{[A_{50}]_3 + [A]}$$

The potency ($[A_{50}]$) of A and the maximum effect (α) can vary in each unit. In the simulations, assays are assumed to consist of (I) one functional unit ($[A_{50}]_1 = 1$ nM, $\alpha_1 = 0.5$), (II) two functional units with $\log[A_{50}]$ values separated by 2 log units ($[A_{50}]_1 = 0.3$ nM, $[A_{50}]_2 = 100$ nM) and equal contributions to the total effect ($\alpha_1 = \alpha_2 = 0.5$), (III) three functional units with $\log[A_{50}]$ values separated by 1.5 log unit intervals ($[A_{50}]_1 = 1$ nM, $[A_{50}]_2 = 30$ nM, $[A_{50}]_3 = 1$ μM) and unequal contributions to the total effect ($\alpha_1 = 0.20$, $\alpha_2 = 0.20$, $\alpha_3 = 0.60$).

Table 1 Analysis of cholecystokinin octapeptide (CCK-8) and pentagastrin E/[A] curves on the guinea-pig gall bladder assay

		logistic curve-fitting parameters[3]		
	n	$\log[A_{50}]$	p	α (force:g)
CCK-8	4	−8.63 ± 0.13	0.63 ± 0.05	0.42 ± 0.12
Pentagastrin	4	−5.38 ± 0.11	0.87 ± 0.18	0.16 ± 0.05[1]

	Agonism model-fitting[2]	
	pK_A	τ
Pentagastrin	4.86 ± 0.52	0.34 ± 0.24

[1]Significantly different from the value of α for the CCK-8 E/[A] curve, $P < 0.05$. [2]CCK-8 was assumed to be a high efficacy agonist in the assay to permit estimation of pK_A and τ values for pentagastrin (see text for details). [3]See Methods for details.

containing CCK_A-receptors (Williams *et al.*, 1978).

Although pentagastrin produced concentration-dependent increases in tension, it was 10000 fold less potent than CCK-8 and the maximum of the pentagastrin E/[A] curve (α) was significantly less than that obtained with CCK-8 (Figure 2a and Table 1). To determine whether pentagastrin was acting as a partial agonist at CCK_A-receptors, two further experiments were performed. First, pentagastrin E/[A] curves were obtained in the presence of a concentration (0.1 μM) of the selective CCK_A-receptor antagonist, devazepide, which is ~1000 fold higher than its K_B at CCK_A-receptors but ~2.5 fold lower than its reported K_B at CCK_B-receptors. Second, CCK-8 E/[A] curves were obtained in the absence and presence of 100 μM pentagastrin which was pre-incubated for 30 min.

The results (Figure 2b) were consistent with pentagastrin behaving as a partial agonist at CCK_A-receptors; that is, devazepide totally abolished the response to pentagastrin and pentagastrin produced a small (1.13 ± 0.18 log unit) significant shift of the CCK-8 E/log[A] curve in quantitative agreement with expectations for the interaction between a low efficacy and high efficacy agonist competing for a common receptor (Barlow *et al.*, 1967). This conclusion was supported by the results of directly-fitting the individual pentagastrin E/[A] data to the model of agonism described by Black & Leff (1983) (see Methods). A good fit was obtained, as judged-by-eye, and estimates were made of the equilibrium dissociation constant, K_A, and the efficacy parameter, τ, for pentagastrin at the CCK_A-receptor (Table 1 and Figure 2a).

The model fitting was only possible by making the, as yet untestable, assumption that CCK-8 was a high efficacy agonist in the assay. Thus, the value of α for CCK-8 could be taken to be equal to the model parameter for the maximum possible agonist effect in the system (E_M) and the midpoint slope parameter (p) of the CCK-8 concentration-effect curve taken to be equal to the midpoint slope of the transducer function (n). Importantly, the pK_B (5.10 ± 0.18, d.f. = 7), estimated for pentagastrin from the single shift competition experiment using CCK-8 as agonist, was not significantly different from the equilibrium dissociation constant value ($pK_A = 4.86 \pm 0.52$, d.f. = 13) estimated by the analysis of the agonism produced by pentagastrin.

Therefore, the presence of potentially-confounding responses due to activation of CCK_B-receptors could be provisionally ruled-out.

Analysis of competitive antagonism

Devazepide, loxiglumide and lorglumide produced concentration-dependent inhibition of the CCK-8-induced contractions resulting in parallel, rightward, displacement of the E/log[A] curves. Subsequent analysis of the $\log[A_{50}]$ values indicated that the compounds behaved as simple competitive antagonists over the range of concentrations used and pK_B values were estimated (Table 2 and Figure 3).

Combined dose-ratio analysis

The combined dose-ratio analysis experiments were designed to test whether the three antagonists, the glutamic acid derivatives (lorglumide and loxiglumide) and the benzodiazepine derivative (devazepide) acted syntopically at the CCK_A-receptor. The optimum discrimination between the multiplicative and additive models is achieved, theoretically, when large dose-ratios are used in the test. However, in practice, we were restricted to a maximum shift of 2–2.5 log units of the CCK-8 E/[A] curve by the limited solubility of CCK-8 itself. Therefore, antagonist concentrations were chosen which were predicted to produce log dose-ratios of approximately 1.7. Thus, if addition of dose-ratios occurred then the predicted combined log dose-ratio of 2 would have been fully-quantifiable. Clearly, if the dose-ratios multiplied then the CCK-8 E/[A] curves would have been displaced to

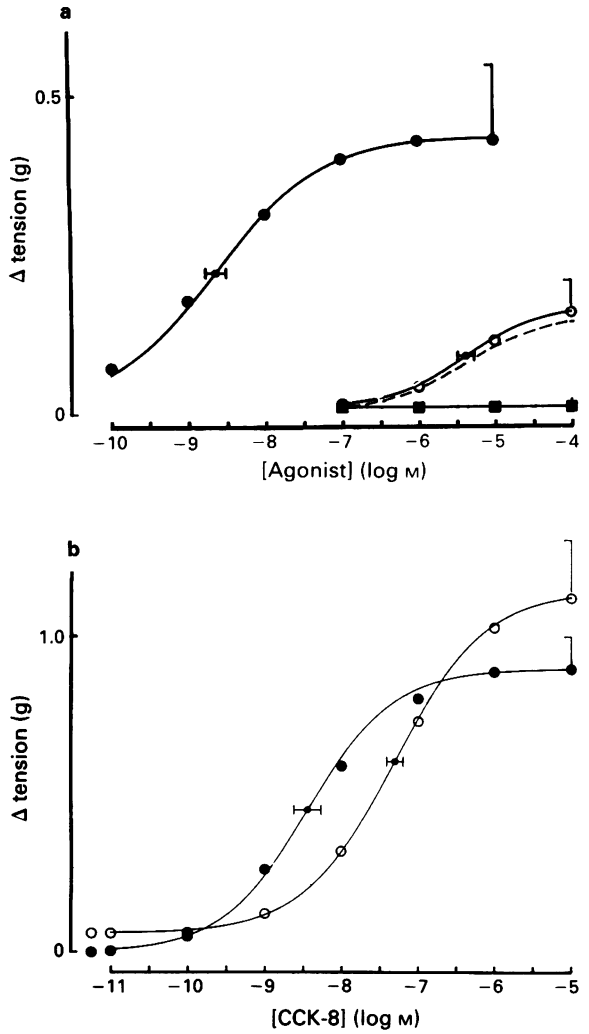

Figure 2 (a) E/[A] curves obtained using cholecystokinin octapeptide (CCK-8) (●), pentagastrin (○) and pentagastrin in the presence of 100 nM L-364718 (■) in the guinea-pig gall bladder assay. Data from individual preparations ($n = 4$) were fitted to a general logistic function and the midpoint slope location ($\log[A_{50}]$) and upper asymptote (α) of the curves expressed as mean ± s.e.mean. The hatched line shown superimposed on the pentagastrin E/[A] data was drawn using the parameters estimated by fitting the Black & Leff (1983) model of agonism (see text for details). (b) CCK-8 E/[A] curves in the absence (●) and presence (○) of 100 μM pentagastrin preincubated for 30 min.

Table 2 Analysis of cholecystokinin octapeptide (CCK-8) antagonist interactions on the guinea-pig gall bladder assay

Compound	*b* (s.e.)	pK_B (s.e.)	*n*
Lorglumide	1.05 (0.23)	7.59 (0.21)	64
Loxiglumide	1.27 (0.16)	7.07 (0.21)	64
Devazepide	1.19 (0.09)	9.98 (0.13)	22

such an extent (log dose-ratio = 3.4) that only threshold CCK-8 responses would have been visible. The data obtained in both experiments were consistent with the additive, syntopic, model and allowed rejection of the multiplicative, allotopic, model (Figure 4 and Table 3). Thus, lorglumide was concluded to be acting syntopically with devazepide which, in turn, was found to act syntopically with loxiglumide.

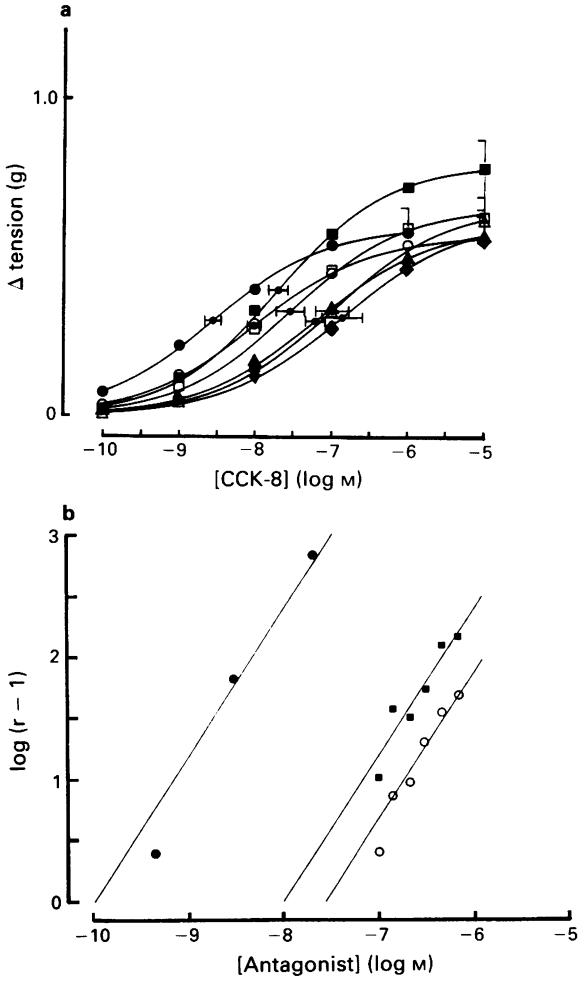

Figure 3 Analysis of competitive antagonism: (a) cholecystokinin octapeptide (CCK-8) E/[A] curves ($n = 6/8$ ± s.e.mean) in the guinea-pig gall bladder assay in the absence (●) and presence of (○) 0.4, (■) 0.6, (□) 1, (▲) 1.5, (△) 2.5, (◆) 4 μM loxiglumide. (b) Schild plots for the interaction between CCK-8 and devazepide (●), loxiglumide (■) and lorglumide (□) on the guinea-pig gall bladder assay.

Discussion

The primary aim of the study was to determine if the benzodiazepine derivative, devazepide, and the glutamic acid derivatives, loxiglumide and lorglumide, act syntopically and competitively at CCK_A-receptors in the guinea-pig gall bladder. Although this preparation has been previously described (La Morte *et al.*, 1981), in practice, we found it was necessary to improve the existing techniques for CCK_A-receptor bioassay. We wanted to apply models of agonism and antagonism, based on the applicability of the Law and Mass Action to the ligand-receptor interactions. These require for simplicity that measurements of effect are made when the agonist response achieves a clearly-defined, sustained plateau. This steady-state condition is usually assumed to indicate an underlying equilibrium condition at the receptors.

In addition to improving the signal-to-noise ratio and reducing spontaneous activity in the tissues to allow accurate, response measurement, the technical changes provided the bonus of increasing the apparent potency of the CCK-8. This allows the behaviour of the antagonists to be studied over a wider range of concentrations, with the E/[A] curves fully-defined, than would have otherwise been possible due to the limited solubility and cost of the peptide agonist, CCK-8.

No evidence for CCK_B-receptors was found in the im-

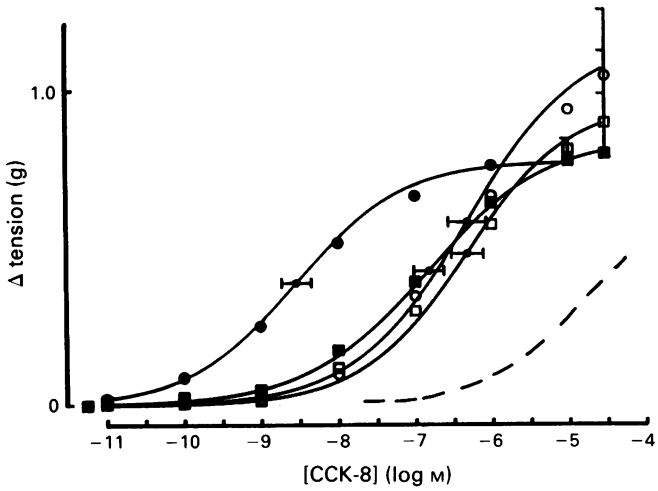

Figure 4 Combined dose-ratio analysis: cholecystokinin octapeptide (CCK-8) E/[A] curves in the absence (●) and presence of 6 nM devazepide (○), 1 μM loxiglumide (■) and a combination of 6 nM devazepide and 1 μM loxiglumide (□). The dashed line shows the location of the CCK-8 E/[A] curve which was predicted by assuming that the antagonists acted independently.

Table 3 Combined dose-ratio analysis

B C (1 μM)	*Devazepide* (6 nM) + *lorglumide* (1 μM)	*Devazepide* (6 nM) + *loxiglumide*
Observed log r_B	1.68	1.20
Observed log r_C	2.18	1.84
Multiplication:		
Expected log $r_{(B+C)}$	3.87	3.04
Addition:		
Expected log $r_{(B+C)}$	2.30	1.92
Observed log $r_{(B+C)}$	2.19	1.99
$S_A \pm$ s.e.	0.11 ± 0.26	0.01 ± 0.21
$S_M \pm$ s.e.	1.68 ± 0.42*	1.14 ± 0.30*

*Significantly different from zero, $P < 0.05$.

proved assay and it was shown that the benzodiazepine derivative, devazepide, and the glutamic acid derivatives, loxiglumide and lorglumide, behaved syntopically and competitively at CCK_A-receptors. From a medicinal chemistry point of view, this result suggests that a molecular model of the CCK_A-receptor requires receptor binding homology for these different chemical structures and the peptide hormone, CCK-8, itself. In addition, we did not find any evidence for CCK_A-receptor heterogeneity as judged by the finding that the CCK-8 E/[A] curves were symmetrical and monotonic in the absence and presence of the antagonists and that the Schild plots were linear over a wide range of antagonist, and, therefore, agonist concentrations.

Recently, Maubach *et al.* (1991) published, in abstract form, data from the analysis of the interactions between, *inter alia*, CCK-8 and two of the antagonists used in this study, devazepide and lorglumide, in an assay also prepared from the guinea-pig gall bladder. In their study, a Schild plot slope significantly greater than unity was obtained with devazepide. The CCK-8 E/[A] curves, in both the absence and presence of the antagonists, were referred to as 'extended' because increases in tension were obtained over 5 log cycles of CCK-8 concentration. Maubach *et al.* (1991) concluded that this was preliminary evidence for CCK_A-receptor heterogeneity. Clearly, our results do not support this view. There are several obvious technical differences between their studies and ours; they incubated antagonists for 30 min rather than 60 min; their experiments were conducted at 37°C whereas we used 29°C; consecutive CCK-8 E/[A] curves were obtained on each preparation rather than a single one; the assay consisted of half gall bladders rather than short, thin strips. Our experience with long strips of tissue suggests that CCK-8 E/[A] curves could be flat with associated high variance if the muscle was taken from a relatively large area of the gall bladder (Figure 1).

We have considered one explanation for these 'extended' curves which may have general applicability to the development and interpretation of other bioassays. Regional variation of hormone receptor concentration is recognised within physiological structures such as the urinary bladder (Taira, 1972). The operational model of agonism (Black & Leff, 1983), in common with other models (Furchgott, 1966), predicts that this variation would have the effect of changing the potency of high efficacy agonists. It was possible to simulate the various profiles of 'extended' CCK E/[A] curves shown in Figure 1 by assuming that the assays prepared from larger pieces of muscle contain several operational units of muscle. Thus, although the receptors in the units are the same, each unit has a different sensitivity to the agonist. The total effect measured in an assay was assumed to be given by the summation of the effects produced by the individual units (Figure 1b). In terms of this model, it is as though, by choosing to use small strips of muscle taken from the central section of the gall bladder, that we have selected a homogeneous muscle unit set with high CCK_A-receptor concentration, so that potent, monotonic CCK-8 E/[A] curves were obtained.

We gratefully acknowledge the skilled technical assistance of Alexander Gerskowitch and Matthew Wilson.

References

BARLOW, R.B., SCOTT, N.C. & STEPHENSON, R.P. (1967). The affinity and efficacy of onium salts on the frog rectus abdominis. *Br. J. Pharmacol.*, **31**, 18–196.

BLACK, J.W., GERSKOWITCH, V.P., LEFF, P. & SHANKLEY, N.P. (1986). Analysis of competitive antagonism when this property occurs as part of a pharmacological resultant. *Br. J. Pharmacol.*, **89**, 547–555.

BLACK, J.W. & LEFF, P. (1983). Operational models of pharmacological agonism. *Proc. R. Soc. B.*, **220**, 141–162.

BLACK, J.W., LEFF, P. & SHANKLEY, N.P. (1985a). Further analysis of anomalous pK_B values for histamine H_2-receptor antagonists on the isolated mouse stomach assay. *Br. J. Pharmacol.*, **86**, 581–587.

BLACK, J.W., LEFF, P., SHANKLEY, N.P. & WOOD, D. (1985b). An operational model of pharmacological agonism: the effect of E/[A] curve shape on agonist dissociation constant estimation. *Br. J. Pharmacol.*, **84**, 561–581.

BLACK, J.W. & SHANKLEY, N.P. (1985). The isolated stomach preparation of the mouse: a physiological unit for pharmacological analysis. *Br. J. Pharmacol.*, **86**, 571–579.

BOCK, M.G., DIPARDO, R.M., EVANS, B.E., RITTLE, K.E., WHITTER, W.L., VEBER, D.F., ANDERSON, P.S. & FREIDINGER, R.M. (1989). Benzodiazepine gastrin and brain cholecystokinin receptor antagonists. *J. Med. Chem.*, **32**, 13–16.

DIXON, W.J. (1990). ed: *BMDP Statistical Software*. Berkeley, Los Angeles & London. University of California Press.

EVANS, E., RITTLE, K.E., BOCK, M.G., DIPARDO, M., WHITTER, W.L., VEBER, D.F., ANDERSON, P.S. & FREIDINGER, R.M. (1986). Design of potent, orally effective nonpeptidal antagonists of the peptide hormone cholecystokinin. *Proc. Natl. Acad. Sci. U.S.A.*, **83,** 4918–4922.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. *Handb. Exp. Pharmakol.*, **33,** 283–335.

LOTTI, V.J., CHANG, R.S.L., KLING, P.J. & CERINO, D.J. (1986). Evidence that cholecystokinin octapeptide (CCK-8) acts as a potent, full agonist on gastrin receptors for acid secretion in the isolated mouse stomach: lack of antagonism by the specific CCK antagonist asperlicin. *Digestion*, **35,** 170–174.

LOTTI, V.J. & CHANG, R.S.L. (1989). A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L365260. *Eur. J. Pharmacol.*, **162,** 273–280.

MAKOVEC, F., BANI, M., CEREDA, R., CHISTE, R., PACINI, M.A., REVEL, L., ROVATI, L.A. & SETNIKAR, I. (1987). Pharmacological properties of lorglumide as a member of a new class of cholecystokinin antagonists. *Arz.-Forsch.*, **37,** 1265–1268.

MAUBACH, K., PATEL, M. & SPRAGGS, C.F. (1991). Interaction of gastrin/cholecystokinin agonists and antagonists on guinea-pig gall bladder. *Br. J. Pharmacol.*, **104,** 142P.

LA MORTE, W.W., HINGSTON, S.J. & WISE, W.E. (1981). pH-dependent activity of H_1- and H_2-histamine receptors in guinea-pig gall bladder. *J. Pharmacol. Exp. Ther.*, **217,** 638–644.

NIEBER, K., MILENOV, K., RAKOVSKA, A., HENKLEIN, P. & OEHME, P. (1988). Responses of guinea-pig gastric, ileal and gall bladder smooth muscle to desamino-cholecystokinin octapeptide (CCK 7). *Meth. Find. Exp. Clin. Pharmacol.*, **10,** 513–520.

PATON, W.D.M. & RANG, H.P. (1965). The uptake of atropine and related drugs by intestinal smooth muscle of the guinea pig in relation to acetylcholine receptors. *Proc. R. Soc. B.*, **183,** 1–44.

SETNIKAR, I., BANI, M., CEREDA, R., CHISTE, R., MAKOVEC, F., PACINI, M.A., REVEL, L., ROVATI, L.C. & ROVATI, L.A. (1987). Pharmacological characterisation of a new potent and specific nonpolypeptide cholecystokinin antagonist. *Arz.-Forsch.*, **37,** 703–707.

SHANKLEY, N.P., BLACK, J.W., GANELLIN, C.R. & MITCHELL, R.C. (1988). Correlation between log $P_{OCT/H20}$ and pK_B estimates for a series of muscarinic and histamine H_2-receptor antagonists. *Br. J. Pharmacol.*, **94,** 264–274.

TAIRA, N. (1972). The autonomic pharmacology of the bladder. *Annu. Rev. Pharmacol.*, **12,** 197–208.

WILLIAMS, J.A., KORC, M. & DORMER, R.L. (1978). Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. *Am. J. Physiol.*, **235,** E517–E524.

WOODRUFF, G.N. & HUGHES, J. (1991). Cholecystokinin antagonists. *Annu. Rev. Pharmacol. Toxicol.*, **31,** 469–501.

(Received November 20, 1991
Revised January 7, 1992
Accepted January 13, 1992)